

AMENDMENTS

Listing of the Claims:

The following listing of claims replaces all previous listing or version thereof:

1-49. (canceled)

50. (currently amended) A method of analyzing an analyte collected on a membrane a fluid sample comprising:

passing one milliliter or less of a fluid sample through a membrane-based flow sensor assembly comprising across a membrane ~~that rest on a membrane support configured to maintain the membrane in a substantially planar orientation,~~ wherein the fluid sample comprises an analyte lymphocytes that are at least partially retained by the membrane;

adding a visualization agent to material contacting the lymphocytes collected on the membrane ~~when the fluid sample is passed across the membrane with a visualization agent;~~

collecting an image of the collected material lymphocytes and analyzing the collected lymphocytes on the membrane using white light, at a first wavelength of light, a second wavelength of light, and a third wavelength of light, wherein the analyte comprises a color corresponding to the first wavelength of light;

forming a first mask corresponding to an image of the collected material at the second wavelength of light;

forming a second mask corresponding to an image of the collected material at the third wavelength of light; and

subtracting the first mask and the second mask from the image of the collected material in white light.

51. (currently amended) The method of claim [[50]]95, wherein the wavelengths of light are selected from the group consisting of red, blue and green.

52. (currently amended) The method of claim [[50]]96, wherein the collecting the image data and forming the masks is performed by a computer.

53. (currently amended) The method of claim [[50]]96, further comprising determining the amount of analyte CD4⁺ lymphocytes present on the membrane by analysis of the image resulting from subtracting the first mask and the second mask from the image of the collected material lymphocytes in white light.

54. (previously presented) The method of claim 50, wherein the images are collected using a digital detection device.

55-68. (canceled)

69. (canceled)

70-80. (cancelled)

81. (canceled)

82. (canceled)

83. (currently amended) The method of claim 50, further comprising:
passing a background fluid across the porous membrane;
detecting an image of matter captured on the porous membrane after passing the background fluid through the porous membrane; and
cleaning the surface of the porous membrane;
comparing the image of matter lymphocytes captured on the porous membrane after passing the sample fluid containing one or more analytes lymphocytes through the membrane to the image of matter captured on the porous membrane after passing the background fluid through the porous membrane.

84. (previously presented) The method of claim 50, wherein the images are collected using a detector, and wherein a programmable controller is coupled to the detector.

85. (canceled)

86. (currently amended) The method of claim 50, wherein the visualization agent comprises a stain label, wherein the stain label is configured to emit light only in a specified portion of the visible spectrum.

87. (currently amended) The method of claim ~~[[50]]96~~, wherein the first mask is a binary mask.

88. (currently amended) The method of claim ~~[[50]]96~~, wherein the second mask is a binary mask.

89. (currently amended) The method of claim ~~[[50]]96~~, wherein the visualization agent comprises a stain label, and wherein further the stain label is configured to emit light only in a green portion of the visible spectrum, and wherein the second wavelength of light comprises a blue portion of the visible spectrum, and wherein the third wavelength of light comprises a red portion of the visible spectrum, and wherein subtracting the first mask and the second mask from the image of the collected material-lymphocyte in white light comprises isolating the matter on the membrane that only emits light in the green portion of the visible spectrum.

90. (currently amended) The method of claim ~~[[69]]50~~, wherein the images are collected using a CCD detector.

91. (currently amended) The method of claim ~~[[69]]50~~, wherein the images are collected using a CCD detector coupled to a microscope.

92. (canceled)

93. (canceled)

94. (canceled)

95. (new) The method of claim 50, wherein the image is collected using white light, at a first wavelength of light, a second wavelength of light, and a third wavelength of light.

96. (new) The method of claim 51, further comprising forming a first mask corresponding to an image of the collected material at the second wavelength of light; forming a second mask

corresponding to an image of the collected material at the third wavelength of light; and subtracting the first mask and the second mask from the image of the collected lymphocytes in white light.

97. (new) The method of claim 50, wherein the fluid sample is a blood sample.
98. (new) The method of claim 86, wherein the label is a fluorescent label.
99. (new) The method of 50, wherein the visualization agent comprises an anti-CD4 antibody.